UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

Septem 23, 2014

MEMORANDUM

SUBJECT: Acute Toxicity Review for EPA Reg. No.: 1677-EUT
DP Barcode: D421076

To: Karen P. Hicks, Team Lead
Chemistry and Toxicology Team (CTT)
Product Science Branch (PSB)
Antimicrobials Division (AD; 7510P)

From: Jenny J. Tao
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division

Through: Ian Blackwell, Biologist (Peer Reviewer)
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division

I.B.

Karen P. Hicks, Team Lead
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division

Applicant: Ecolab, Inc.

FORMULATION FROM LABEL:

PC Code	Active Ingredient(s)	% by wt.
000595	Hydrogen Peroxide	0.045%
047501	Isopropanol	10.89%
069105	Alkyl(50% C_{14}, 40% C_{12}, 10% C_{16}) dimethyl benyl ammonium chloride	0.016%
069165	Octyl decyl dimethyl ammonium chloride	0.012%
069208	Didecyl dimethyl ammonium chloride	0.007%
069166	Dioctyl dimethyl ammonium chloride	0.005%
	Inert Ingredient(s):	89.025 %
	Total:	100.0 %

BACKGROUND:

The registrant, Ecolab, Inc., submitted a "Me-too" application, DrySan Duo (KX-6230), a new end use product used as a cleaner and food-contact and non-food contact sanitizer, containing hydrogen peroxide (0.045%), isopropanol (10.89%), alkyl(50% C_{14}, 40% C_{12}, 10% C_{16}) dimethyl benyl ammonium chloride (0.016%), Octyl decyl dimethyl ammonium chloride (0.012%), didecyl dimethyl ammonium carbonate (0.007%), and dioctyl dimethyl ammonium chloride (0.005%) as the active ingredients (a.i.).

The acute toxicity data of the application include acute oral, dermal, and inhalation toxicities, primary dermal and eye irritation, and dermal sensitization (Local Lymph Node Assay in Mice [LLNA-BrdU ELISA]).

RECOMMENDATIONS:

1. DrySan Duo (KX-6230) is Category IV for acute oral, inhalation, and dermal toxicity and the eyes. It is moderately irritating to the skin but is not a skin sensitizer.

2. Precautionary language for the skin, in addition to a signal word of "CAUTION", needs to be included in the label due to the irritation of the product.

3. No precautionary language is required for acute oral, dermal, and inhalation toxicities and the eyes; however, Category III labeling could be chosen, if desired so.

The Acute Toxicity Profile for EPA Reg. No.: 1677-EUT is currently:

Study	MRID Number	Toxicity Category	Status
Acute Oral Toxicity	49363220	IV	Acceptable
Acute Dermal Toxicity	49363221	IV	Acceptable
Acute Inhalation Toxicity	49363223	IV	Acceptable
Primary Eye Irritation	49363219	IV	Acceptable
Primary Skin Irritation	49363218	III	Acceptable
Dermal Sensitization	49363222	Not a sensitizer	Acceptable

LABELING

4) REQUIRED PRECAUTIONARY LABELING:
"Keep Out of Reach of Children"
CAUTION: Causes moderate skin irritations. Avoid contact with skin or clothing. Wear long-sleeved shirt and long pants, socks, shoes and gloves. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet.

5) REQUIRED FIRST AID STATEMENTS:

If on skin:

- Take off contaminated clothing.
- Rinse skin immediately with plenty of water for 15-20 minutes.
- Call a poison control center or doctor for treatment advice.

  - Have the product container or label with you when calling a poison control center or doctor or going for treatment.
  - For emergency information on (product, use, etc.), call the National Pesticides Information Center at 1-800-858-7378, 6:30 AM to 4:30 PM Pacific time (PT), seven days a week. During other times, call the poison control center 1-800-222-1222.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (OPPTS 870.1100)
UP – DOWN Procedure (UDP)

Product Manager:	Team 33	**Reviewer**: Jenny J. Tao	
MRID No.:	49363220	**Study Completion Date**: 24 Februry, 2014 **Lab Project No.**: MB 13-22220.01	

Testing Laboratory: MB Research Laboratories
1765 Wentz Road, P. O. Box 178
Spinnerstown, PA 18968
Author: Blair, Y.
Sponsor: Ecolab, Inc.
655 Lone Oak Drive
Eagan, MN 55121

Quality Assurance (40 CFR §160.12): Quality Assurance and Data Confidentiality statements were included. A statement of Good Laboratory Practice (GLP) was included stating that the study meets the requirements of the EPA 40 CFR Part 160 and 792 (FIFRA) and current OECD Principles of GLP (as revised in 1997): ENV/MC/CHEM (98)17, OECD, Paris, 1998.

Test Material: KX-6230 (purity ≥99.9% (wt/wt) a.i.: 0.0415% Quat, 0.0452% H_2O_2, and 10.90% isopropyl alcohol), Lot # P080731, clear colorless liquid

Dosage: Limit Test: 5000 mg/kg

Species: Rat/Sprague-Dawley derived albino
Sex: 3 Female, nulliparous and non-pregnant
Age: young adult (approx. 8 – 12 weeks old)
Weight: 218-225 grams at experimental start
Source: Charles River, Stone Ridge, NY
Housing: animals were singly housed in suspended stainless steel cages with mesh floors
Diet: PMI Rat Chow (Diet #5012)
Water: water was available *ad libitum*
Environmental Conditions: Temperature Range: 68-79 °F
Humidity Range: 30-70%
Photoperiod: 12- hour light/dark cycle
Acclimation: at least 5 days

Study experimentation (in-life) dates: Start: 04 December, 2013; End: 26 December, 2013

Animal assignment and treatment: Initially one fasting animal was given a single gavage dose of 5000 mg/kg KX-6230. That animal survived; therefore, two additional fasting animals were dosed at 5000 mg/kg KX-6230. All test animals were observed for toxicity and pharmacological effects at postdosing of 15 minutes, 1, 2, and 4 hours and once daily for 14 days and twice daily for mortality. Individual body weights were recorded immediately pre-dosing and again on study days 7 and 14. Surviving animals were euthanized via CO_2 inhalation after the 14-day observation period. Gross necropsies were performed on all animals.

Statistics: The Limit LD_{50} was based on the outcome of three or more animals survived at the dose of 5000 mg/kg.

Conclusion:

1. **The acute oral LD_{50} was determined to be >5000 mg/kg of body weight in female rats.**

2. **Toxicity Category**: IV **Classification**: Acceptable

Procedure (Deviations from OPPTS 870.1100): The room temperature and the humidity were fluctuated between 67.2 and 72.2 ^{0}F and 0.0 to 65.4%, respectively, during the time period of the study. However, these fluctuations happened gradually and the temperature remained relatively consistent while the humidity intermittently deviated from the Guideline recommended ranges. The deviation of the room temperature was negligible; overall, these fluctuations did not affect the study result, as determined by the Study Diractor.

Amendment to Final Protocol: None.

Results:

Dosing Sequence	Animal No.	Dose Level (mg/kg bw)	Short-term Outcome	Long-term Outcome	Body Weight (g)		
					Day 0	Day 7	Day 14
1	1	5,000	S	S	225	271	282
2	2	5,000	S	S	220	241	246
3	3	5,000	S	S	218	245	249

S – Survival

Observations:

All animals survived, gained body weight, and appeared active and healthy during the study. There were no signs of clinical toxicity, adverse pharmacological effects, or abnormal behavior.

Gross Necropsy:

No gross abnormalities were observed for the animals when necropsied at the conclusion of the 14-day observation period.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (OPPTS 870.1200)

Product Manager: Team 33 **Reviewer**: Jenny J. Tao

MRID No.: 49363221 **Study Completion Date**: 24 Februry, 2014
Lab Project No.: MB 13-22220.02

Testing Laboratory: MB Research Laboratories
1765 Wentz Road, P. O. Box 178
Spinnerstown, PA 18968
Author: Blair, Y.
Sponsor: Ecolab, Inc.
655 Lone Oak Drive
Eagan, MN 55121

Quality Assurance (40 CFR §160.12): Quality Assurance and Data Confidentiality statements were included. A statement of Good Laboratory Practice (GLP) was included stating that the study meets the requirements of the EPA 40 CFR Part 160 and 792 (FIFRA) and current OECD Principles of GLP (as revised in 1997): ENV/MC/CHEM (98)17, OECD, Paris, 1998.

Test Material: KX-6230 (purity ≥99.9% (wt/wt) a.i.: 0.0415% Quat, 0.0452% H_2O_2, and 10.90% isopropyl alcohol), Lot # P080731, clear colorless liquid

Dosage: Limit Test: 5000 mg/kg

Species: Rabbit; New Zealand, albino
Sex: 5 Males and 5 nulliparous and non-pregnant Females
Age: young adult (at least 12 weeks old)
Weight: ♂: 2.4-2.6 kg; ♀: 2.7-3.0 kg at experimental start
Source: Covance Research Products, Inc., Denver, PA
Housing: animals were singly housed in suspended stainless steel cages with mesh floors
Diet: PMI Rabbit Chow (Diet #5321)
Water: water was available *ad libitum*
Environmental Conditions: Temperature Range: 61-72 °F
Humidity Range: 30-70%
Photoperiod: 12- hour light/dark cycle

Acclimation: at least 5 days

Study experimentation (in-life) dates: Start: 03 December, 2013; End: 17 December, 2013

Animal assignment and treatment: Approximately 24 hours prior to treatment, test animals were prepared by clipping the dorsal area and the trunk, which began at the shoulders and extended to the hipbone and half way down the flank of each side of the animal. On the day of treatment, a single dose of 5000 mg/kg KX-6230 was dermally applied to ten healthy animals (5 males and 5 females) over an area of approximately 10% of the body surface; then wrapped with a piece of porous dressing (semi-occlusive) and secured with non-irritatin tape. After a 24-hour exposure period, the wrappings were removed and the test sites was gently washed with distilled water to remove any residual test material. The test sites were scored for dermal irritation at 24 hours postdosing and on

Day 14 using the Draize scoring method. Ulceration and necrosis or any evidence of tissue destruction were also evaluated. Animals were also observed at 1 and 4 hours postdosing and once daily for 14 days for mortality, clinical signs of toxicity, and pharmacological effects. Body weight was recorded prior to dosing, weekly, and at termination. All rats were euthanized via CO_2 inhalation on day 14 and examined for gross pathology.

Statistics: The LD_{50} was estimated based on mortality occuring during the study.

Summary:

1. **Acute Dermal LD_{50} (mg/kg)**: >5,000 mg/kg bw for both male and female rats

2. **Toxicity Category**: IV **Classification**: Acceptable

Procedure (Deviations from 870.1200): The room temperature and the humidity were fluctuated between 54.6 and 71.8 ^{0}F and 0.0 to 62.0%, respectively, during the time period of the study. However, the fluctuation in humidity occured gradually during which the temperature remained relatively consistent. Overall, the fluctuations did not affect the study result, as determined by the Study Diractor.

Amendment to Final Protocol: None.

Results:

Reported Mortality

Dose Level (mg/kg bw)	Number Dead/Number Tested (mg/kg bw)			Body Weight (mean kg±S.D.)		
	Males	Females	Total	Day 0	Day 7	Day 14
5,000	0/5	0/5	0/10	♂: 2.6 ± 0.1 ♀: 2.9 ± 0.1	♂: 2.6 ± 0.1 ♀: 3.0 ± 0.2	♂: 2.8 ± 0.1 ♀: 3.2 ± 0.2

Dermal Observations (Draize scoring)

		Erythema					Edema				
		0	1	2	3	4	0	1	2	3	4
M	24-hr	0/5	2/5	3/5	0/5	0/5	4/5	0/5	1/5	0/5	0/5
	Day-14	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
F	24-hr	0/5	2/5	3/5	0/5	0/5	2/5	1/5	1/5	1/5	0/5
	Day-14	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Observations:

All animals survived exposure to the test substance and gained weight by study termination. Dermal irritation, including erythema and edema, was observed at the dose site of some of the animals at 24 hours but were absent by Day 14. There were no other signs of clinical toxicity or adverse pharmacologic effects. No gross necropsy findings.

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (OPPTS 870.1300)
(WHOLE BODY EXPOSURE)

Product Manager:	Team 33	**Reviewer**: Jenny J. Tao	
MRID No.:	49363223	**Study Completion Date**: 08 April, 2014 **Lab Project No.**: MB 13-22220.05	
Testing Laboratory:	MB Research Laboratories 1765 Wentz Road, P. O. Box 178 Spinnerstown, PA 18968		
Author:	Blair, Y.		
Sponsor:	Ecolab, Inc. 655 Lone Oak Drive Eagan, MN 55121		

Quality Assurance (40 CFR §160.12): Quality Assurance and Data Confidentiality statements were included. A statement of Good Laboratory Practice (GLP) was included stating that the study meets the requirements of the EPA 40 CFR Part 160 and 792 (FIFRA) and current OECD Principles of GLP (as revised in 1997): ENV/MC/CHEM (98)17, OECD, Paris, 1998.

Test Material: KX-6230 (purity ≥99.9% (wt/wt) a.i.: 0.0415% Quat, 0.0452% H_2O_2, and 10.90% isopropyl alcohol), Lot # P080731, clear colorless liquid

Concentration: 2.18 mg/L

Species:	Rat/Sprague-Dawley derived albino
Sex:	5 males and 5 nulliparous and non-pregnant females
Age:	young adult (approx. 10 – 11 weeks old)
Weight:	365-376 grams for males and 260-285 for females at experimental start
Source:	Charles River, Stone Ridge, NY
Housing:	animals were singly housed in suspended stainless steel cages with mesh floors
Diet:	PMI Rat Chow (Diet #5012)
Water:	water was available *ad libitum*
Environmental Conditions:	Temperature Range: 68-79 °F Humidity Range: 30-70% Photoperiod: 12- hour light/dark cycle
Acclimation:	at least 5 days

Study Experimentation (in-life) dates: Start: 27 February, 2014; End: 13 Mar, 2014

Animal assignment and treatment: 5 male and 5 female rats were acclimated to the exposure chamber condition for at least 10 minites prior to exposure of the test substance via whole body exposure for a period of 4 hours. All animals were monitored for toxicity and pharmacological effects during the exposure period, one hour after exposure, and once daily thereafter for 14 days. The animals were observed for mortality twice daily during the study. Individual body weights were recorded prior to test substance exposure and on Study Days 7 and 14. Gross necropsies were performed on all animals.

Exposure Conditions: A 100-liter dynamic glass chamber system, divided into 10 non-restraining enclosed compartiment using wire screening, was used during exposure to allow for continuous observation of animals and ensure uniform spatial distribution of aerosolized test substance. One animal was placed into each compartment during the exposure and returned back to its individual housing following exposure.

A vacuum pump was used to generate the airflow to achieve at least 10 air changes per hour, measured by a rotameter (Gilmont B992), to ensure adequate oxygen supply to the animals during the exposure. In order to keep the test atmosphere contained, a slight negative pressure differential to the immediate environment was maintained. During the exposure period (4 hours), room temperature and humidity, airflow (exhaust), and negative pressure were recorded at approximately 30-minute intervals.

The test atmosphere concentration was determined gravimetrically by withdrawing air samples at 5 intervals from the breathing zone of the chamber during the exposure period. Filter papers through which the air samples passed were weighed before and after collection to determine the mass collected. The concentrations (mg/L) were calculated by determining the mean total of the net weight of test substance collected, the collection efficiency (filter weight post airflow ÷ filter weight pre airflow), and the total airflow volume (in titers) during the sampling time period, which was (net weight of test substance collected ÷ collection efficiency) ÷ (total airflow volume) x 1000.

Particle size is measured at least 3 times during the exposure period. Size distribution in term of aerodynamic mass median diameter (MMAD) and geometric standard deviation (GSD) were determined using three-cycle logarithmic probit paper to graph the percent of total particle weight against the cumulative period.

Statistics: A LC_{50} value was estimated based on mortality observed during the study.

Concentration:

Group	Gravimetric Exposure Concentration (mg/L)	Nominal Concentration (mg/L)
1	2.18	69 mg/L

Summary:

1. **Acute Inhalation LC_{50} 4-hr whole-body exposure**: > 2.18 mg/L for male and female rats.

2. **Average MMAD**: 1.65 μm

4. **Toxicity Category**: IV **Classification**: Acceptable

Procedure (Deviations from 870.1300): The room temperature and the humidity were fluctuated from 67.2 to 70.9 ^{0}F and 2.0 to 46.2%, respectively, during the time period of the study 2.0 to 46.2%, which were lower than the recommended temperature and humidity for rats. However, the fluctuation in humidity occured gradually during which the temperature remained relatively consistent. Overall, the fluctuations did not affect the study result, as determined by the Study Diractor.

Results:

Clinical Observations: All animals survived the study. The following clinical signs of toxicity were observed during the exposure period: wetness of the nose/mouth area, test substance coated fur, and closed eyes. Test substance coated fur persisted through Study Day 1 post dosing.

Two females lost body weights and one female didn't gain body weight between Study Day 7 and 14; all other animals gained body weight by study termination.

Gross Necropsy:
Gross necropsy revealed moderate localized hair loss in the neck in one animal; no other observable abnormalities were noted in the other nine animals.

Chamber Atmosphere[1]

Exp. Conc. (mg/L)	MMAD (µm)	GSD[2] (µm)	Cumulative % of Particles < Effective Cutoff Diameter (µm)				
			0.4	0.7	2.1	4.7	5.8
2.18	1.65	2.78	11.89	24.10	53.90	84.76	100.00

[1]Average of three samples.
[2] Square root of 84%/16%.

Chamber Environment during Exposure

Exposure Level (mg/L)	2.18
Chamber Volume (L)	100
Average Total Airflow Volume (Lpm)	Not reported
Air Changes Per Hour	At least 10
Mean Oxygen Content (%)	Not reported
Temperature Range (°F)	67.2-70.9
Relative Humidity Range (%)	2.0-46.2

DATA REVIEW FOR ACUTE EYE IRRITATION TESTING (OPPTS 870.2400)

Product Manager: Team 33
MRID No.: 49363219

Reviewer: Jenny J. Tao
Completion Date: 21 February, 2014
Study No.: MB 13-22220.04

Testing Laboratory: MB Research Laboratories
1765 Wentz Road, P. O. Box 178
Spinnerstown, PA 18968
Author: Hall, D.
Sponsor: Ecolab, Inc.
655 Lone Oak Drive
Eagan, MN 55121

Quality Assurance (40 CFR §160.12): Quality Assurance and Data Confidentiality statements were included. A statement of Good Laboratory Practice (GLP) was included stating that the study meets the requirements of the EPA 40 CFR Part 160 and 792 (FIFRA) and current OECD Principles of GLP (as revised in 1997): ENV/MC/CHEM (98)17, OECD, Paris, 1998.

Test Material: KX-6230 (purity ≥99.9% (wt/wt) a.i.: 0.0415% Quat, 0.0452% H_2O_2, and 10.90% isopropyl alcohol), Lot # P080731, clear colorless liquid

Dosage: 0.1 mL (undiluted)

Species: Rabbit/New Zealand White
Sex: 2 Males and 1 nulliparous and non-pregnant female
Age: young adult
Weight: 2.8-3.4 kg at experimental start
Source: Covance Research Products, Inc., Denver, PA
Housing: animals were singly housed in suspended stainless steel cages with mesh floors
Diet: PMI Rabbit Chow (Diet #5321)
Water: water was available *ad libitum*
Environmental Conditions: Temperature Range: 61-72 °F
Humidity Range: 30-70%
Photoperiod: 12- hour light/dark cycle
Acclimation: at least 5 days

Study Experimentation (in-life) dates: Start: 10 December, 2013; End: 13 December, 2013

Animal assignment and treatment: On the day of treatment, two healthy male rabbits and one nulliparous and non-pregnant healthy female rabbit were examed for any ocular irritation and/or corneal abnormalities. An analgesic, Buprenorphine, was administered subcutaneously between the shoulder blades, at least one hour and no more than four hours before adminitration of the test substance. A dose of 0.1 ml of the test substance was placed into the conjunctival sac of one eye of each rabbit. The lids of the treated eye were held together after instillation for approximately one second to insure adequate distribution of the test material. The untreated contralateral eye served as the control. Both the treated and the control eyes were scored for irritation of the cornea, iris, and conjunctiva using the Draize numerical system at 1, 24, 48, and 72 (±10%) hours post-treatment. Sodium fluorescein dye procedures were used at the 24-hour post-treatment interval. Animals were evaluated for physical signs twice daily for the first three days of post-treatment. Body weight was recorded immediately prior to dosing and at termination. All test rabbits were euthanized via CO_2 inhalation following study termination.

Statistics: No calculation was carried out.

Summary:

1. **Toxicity Category**: IV
2. **Classification**: Acceptable

Procedure (Deviations from 870.2400): The room temperature and the humidity were fluctuated between 68.5 and 71.0^{0}F and 7.5 to 23.8%, respectively, during the time period of the study. However, the fluctuation in humidity occured gradually during which the temperature remained relatively consistent. Overall, the fluctuations did not affect the study result, as determined by the Study Diractor.

Results:
Conjuctival irritation was observed in all of the three eyes treated, cleared in one eye by 24 hours and within 48 hours in the other two eyes. There were no corneal opacity or iritis noted in any of the treated eyes during all obervation periods.

No abnormal physical signs were observed. All test animals lost body weight at the termination of the study.

Individual Scores for Ocular Irritation and Body Weight

Animal #/Sex	Observations	Hours After Treatment				Body Weight	
		1	24	48	72	Pre-dosing	Termination
H6719/♂	I. Corneal Opacity	0	0	0	0	2.8 kg	2.7 kg
	II. Iritis	0	0		0		
	III. Conjunctivae						
	A. Redness	2	1	0	0		
	B. Chemosis	0	0	0	0		
	C. Discharge	0	0	0	0		
H6720/♂	I. Corneal Opacity	0	0	0	0	3.4 kg	3.3 kg
	II. Iritis	0	0	0	0		
	III. Conjunctivae						
	A. Redness	1	0	0	0		
	B. Chemosis	0	0	0	0		
	C. Discharge	2	0	0	0		
H6755/♀	I. Corneal Opacity	0	0	0	0	3.0 kg	2.9 kg
	II. Iritis	0	0	0	0		
	III. Conjunctivae						
	A. Redness	1	1	0	0		
	B. Chemosis	1	0	0	0		
	C. Discharge	2	0	0	0		

DATA REVIEW FOR ACUTE DERMAL IRRITATION TESTING (OPPTS 870.2500)
(Draize Method)

Product Manager: Team 33 **Reviewer**: Jenny J. Tao

MRID No.: 49363218 **Study Completion Date**: 24 Feb, 2014
Lab Study No.: MB 13-22220.03

Testing Laboratory: MB Research Laboratories
1765 Wentz Road, P. O. Box 178
Spinnerstown, PA 18968
Author: Blair, Y.
Sponsor: Ecolab, Inc.
655 Lone Oak Drive
Eagan, MN 55121

Quality Assurance (40 CFR §160.12): Quality Assurance and Data Confidentiality statements were included. A statement of Good Laboratory Practice (GLP) was included stating that the study meets the requirements of the EPA 40 CFR Part 160 and 792 (FIFRA) and current OECD Principles of GLP (as revised in 1997): ENV/MC/CHEM (98)17, OECD, Paris, 1998.

Test Material: KX-6230 (purity ≥99.9% (wt/wt) a.i.: 0.0415% DDAC [Quat], 0.0452% H_2O_2, and 10.90% isopropyl alcohol), Lot # P080731, clear colorless liquid

Dosage: 0.5 ml

Species: Rabbit/New Zealand White
Sex: 2 Males and 1 nulliparous and non-pregnant female
Age: young adult (at least 12-week old)
Weight: 2.5-2.8 kg at experimental start
Source: Covance Research Products, Inc., Denver, PA
Housing: animals were singly housed in suspended stainless steel cages with mesh floors
Diet: PMI Rabbit Chow (Diet #5321)
Water: water was available *ad libitum*
Environmental Conditions: Temperature Range: 61-72 °F
Humidity Range: 30-70%
Photoperiod: 12- hour light/dark cycle
Acclimation: at least 5 days

Study Experimentation (in-life) dates: Start: 3 December, 2013; End: 10 December, 2013

Animal assignment and treatment: The test substance was tested dermally at three sites (approx. 2x3 cm^2 each site) of each animal. Approximately 24 hours prior to treatment, the dorsal area of the trunk of test animal was clipped for a free of hair area. On the day of treatment, a volume of 0.5 ml of KX-6230 (per site) over a 2x3 cm gauze patch was applied dermally onto the three test sites of each test animal. The treatment sites (with the gauze patch) were then secured with non-irritatin tape. The torso of each animal was covered with a piece of porous dressing (semi-occlusive) large enough to cover the dose site with at least 5 cm^2 to spare on all sides of the gauze patch; porous, non-irritating tape was used to encircle the trunk of each treated animal. The treatement patch was

kept in contact with the skin for a 4-hour exposure period; then the wrappings and patches were removed. The test sites was gently washed with distilled water to remove any residual test material. The test sites were scored for dermal irritation at 1, 24, 48, and 72 hours following patch removel and on Day 7 for one animal using the Draize numerical scoring method for erythema and edema. Ulceration and necrosis or any evidence of tissue destruction were also evaluated.

Animals were also observed for clinical signs of toxicity and pharmacological effects at each dermal observation period and for mortality daily. Body weight was recorded prior to dosing and at termination. All rats were euthanized via CO_2 inhalation following study termination.

Statistics: No calculation was carried out.

Summary:

1. **Toxicity Category**: III

2. **Classification**: Acceptable

Procedure (Deviations from 870.2500): The room temperature and the humidity were fluctuated between 54.6 and 71.8 ^{0}F and 3.1 to 62.0%, respectively, during the time period of the study. However, the fluctuation in humidity occured gradually during which the temperature remained relatively consistent. Overall, the fluctuations did not affect the study result, as determined by the Study Diractor.

Results:
Dermal irritation was observed consistently in just one male (H6684) during the study. At 1-hour post exposure, very slight erythema and no edema were exhibited in one male. Slight erythema progressed to well-defined with slight edema in this same male animal at 24- and 48-hour post exposure. Well-dedined erythema persisted in this same male animal until Day 7. No dermal irritation was observed in the other male animal nor in the female animal.

Both male animals gained body weight during the study; the body weight of the female animal remained unchanged. No clinical signs of toxicity and/or pharmacological effects were observed in any of the testing animals during the study.

Under the conditions of this study, KX-6230 was irritating to the skin.

Individual Skin Irritation (Erythema/Edema) Scores and Boday Weight Changes

Animal No.	Sex	Time after Patch Removal					Body Weight Changes		
		1 hour	24 hours	48 hours	72 hours	Day 7	Pretest	72 hours	Day 7
H6709	F	0	0	0	0	N/A	2.8	2.8	N/A
H6683	M	0	0	0	0	N/A	2.8	2.9	N/A
H6684	M	1/0	2/1	2/1	2/0	0/0	2.5	N/A	2.7
Total		1/0	2/1	2/1	2/0	0/0	N/A	N/A	N/A

N/A: not applicable

DATA REVIEW FOR SKIN SENSITIZATION TESTING (OPPTS 870.2600)
(Local Lymph Node Assay [LLNA-BrdU ELISA])

Product Manager: Team 33 **Reviewer**: Jenny J. Tao

MRID No.: 49363222 **Study Completion Date**: 21 Feb, 2014
Lab Study No.: MB 13-22220.26

Testing Laboratory: MB Research Laboratories
1765 Wentz Road, P. O. Box 178
Spinnerstown, PA 18968
Author: Carathers, M.
Sponsor: Ecolab, Inc.
655 Lone Oak Drive
Eagan, MN 55121

Quality Assurance (40 CFR §160.12): Quality Assurance and Data Confidentiality statements were included. A statement of Good Laboratory Practice (GLP) was included stating that the study meets the requirements of the EPA 40 CFR Part 160 and 792 (FIFRA) and current OECD Principles of GLP (as revised in 1997): ENV/MC/CHEM (98)17, OECD, Paris, 1998.

Test Material: KX-6230 (purity ≥99.9% (wt/wt) a.i.: 0.0415% Quat, 0.0452% H_2O_2, and 10.90% isopropyl alcohol), Lot # P080731, clear colorless liquid
Positive Control Material: alpha-Hexylcinnamaldehyde (HCA), 85%, Lot# MKBN8353V, in Acetone/Olive Oil (4:1) (AOO) (25% [v/v] HCA)
Vehicle Control: Acetone/Olive Oil (4:1) (AOO); Fisher Scientific Lot# 124017/Sigma Lot# BCBH9319V

Species:	CBA/J mice, nulliparous and non-pregnant females	
Sex:	Screen (3 groups):	2/group
	Test Group (3 groups):	5/group
	Vehicle (Negative) Control Group:	5
	Positive Control Group:	5
	Naïve Control Group:	5
Age:	Young adult (approx. 8 weeks old)	
Weight:	Screen: 19.1 – 23.7 grams; Main Test: 18.5 – 22.5 grams	
Source:	Jackson Laboratories, Bar Harbor, ME	
Housing:	animals were singly housed in suspended stainless steel cages with mesh floors	
Diet:	PMI Rodent Chow (Diet #5001)	
Water:	water was available *ad libitum*	
Environmental Conditions:	Temperature Range:	68-79 °F
	Humidity Range:	30-70%
	Photoperiod:	12-hour light/12-hour dark cycle
Acclimation:	at least 5 days	

Method: Local Lymph Node Assay (LLNA-BrdU ELISA)

Study Experimentation (in-life) dates: Start: 11 December, 2013; End: 30 December, 2013

Summary:

1. **Based on these findings and on the evaluation system used,** KX-6230 is not considered to be a dermal sensitizer.

2. **Classification**: Acceptable

Procedure (Deviations from 870.2600): The room temperature and the humidity were fluctuated between 66.9 and 71.2 ^{0}F and 0 to 62.0%, respectively, during the time period of the study. However, the fluctuation in humidity occured gradually during which the temperature remained relatively consistent. Overall, the fluctuations did not affect the study result, as determined by the Study Diractor.

Procedure:

Preliminary Dermal Irritation Screen: Prior to the LLNA study, several concentrations of the test substance were evaluated for irritation potential as measured by erythema of the ears. Mice (2 females/group) received one application of the test substance at concentrations of 25% (v/v in AOO), 50% (v/v in AOO), or 100%, on the dorsal surface of each ear once daily for three consecutive days. The test solutions (25 µL/ear) were spread over the entire dorsal surface of each ear in a manner to prevent material loss.

Main Test: Based on the results of the preliminary screen test, concentrations of 25% (v/v in AOO), 50% (v/v in AOO), or 100% were chosen for the main LLNA test, since no local dermal irrition nor overt systemic toxicity was observed at the highest achievable concentration. Five mice per group received one application of the test substance at concentrations of 25% (v/v in AOO), 50% (v/v in AOO), or 100%, on the dorsal surface of each ear once daily for three consecutive days. The test material was applied in the same manner as described above in the screen test. Five mice of each group, treated in the same manner as those in the dosed groups, received Vehicle Control (AOO), 25% (v/v in AOO) HCA Positive Control, or Naïve Control.

All animals were observed once daily throughout the study for clinical signs of toxicity, local dermal irrition at the application site, and mortality. Body weights were measured and recorded on Study Days 1 (immediately prior to dosing) and 6 (prior to sacrifice).

Dermal Scoring: Ears were inspected and thickness measurements were performed on Study Days 1 (immediately prior to dosing), 3 (before the third dose [approximately 48 hours after the first dose]), and 6 (before sacrifice [approximately 120 hours after the first dose and 72 hours after the third dose]).

5-Bromo-2'-Deoxy-Uridine (BrdU) Injections: On Study Day 5, approximately 96 hours following the initial dose and 24 hours prior to sacrifice, all mice received a 500 µL/mouse (10 mg/mL) 5-bromo-2'-deoxy-uridine (BrdU), an analog of thymidine, in Dulbecco's phospate buffered saline (DPBS) intraperitoneal injection. The injected BrdU becomes incorporated into the DNA of proliferating cells, including proliferating nodal lymphocytes.

Lymph Node Assessment by Determination of BrdU Incorporation: Each mouse was euthanized via CO_2 asphyxiation. The auricular lymph nodes located at the bifurcation of the jugular veins were isolated and collected. The auricular lymph nodes were combined for each mouse, and single-cell suspensions of lymph node cells (LNC) were generated in RPMI-10 medium, then permeabilized and denatured in triplicate in a flat-bottom 96-well microplate for measurement of the amount of BrdU incorporation by an enzyme-linked immunosorbent assay (ELISA) kit (Lot# 14457600, Roche Applied Science, Indianapolis, IN).

Using a MicroQuant plate reader (Bio-Tek Instruments), absorbance of each well was measured; then the mean optical density (OD) values ± standard deviation (S.D.) was calculated.

Calculation of Stimulation Index (SI): For *each* mouse, the SI is calculated by dividing the mean OD of each animal by the mean OD of the Vehicle Control group:

$$\text{SI/mouse} = \frac{\text{Mean OD}_{\text{test substance}}}{\text{Mean OD}_{\text{vehicle}}}$$

For the 100% test substance group, the mean OD of the Sham-treated (Naïve) group served as the Control. From the individual animal data, the mean SI ± SD was calculated for each group.

Evaluation:
If a test substance results in a 1.6-fold or greater increase in the mean LNC proliferation relative to that obtained for the Vehicle Control (SI), the test substance is considered to have a positive response. Therefore, a SI ≥ 1.6 for the test substance groups were characterized as sensitizing substances.

Statistical Analysis: The individual animal SI values along with the mean group SI and standard deviation were calculated, for each test group. ANOVA followed by the Students' t-Test was run for comparison between each test substance group to the Vehicle Control group. The 100% test substance group was compared to the Sham-treated (Naive) Control.

Results:

All animals survived the study and were observed normal overall. Non-significant body weight losses (< 2 grams) were observed.

Preliminary Irritation Testing:
Ear thicknesses were measured on Study Day 1 prior to dosing, on Study Day 3 before the third test substance application (48 hours after the initial dose), and on Study Day 6 before sacrifice. As indicated in the Table below, none of the test substance treatments resulted in increases in ear thickness of 25% or more; therefore, the test substance was not considered irritating. Based on these screen results, concentrations of 25% and 50% (v/v in AOO) and 100% of the test substance were selected for the main LLNA test.

Conc. Level	Animal	Ear Thickness (mm)						Mean Ear Thickness (mm)			Change (%)[1]	
		Study Day 1		Study Day 2		Study Day 3		Predoing (Day 1)	48-hr (Day 3)	Study End (Day 6)	Day 3 – Day 1	Day 6 – Day 1
		Left Ear	Roght Ear	Left Ear	Roght Ear	Left Ear	Roght Ear					
25%	1	0.20	0.20	0.20	0.20	0.21	0.22	0.20	0.20	0.21	0.0%	5.0%
	2	0.19	0.20	0.20	0.20	0.20	0.21					
50%	3	0.19	0.21	0.21	0.22	0.21	0.22	0.20	0.21	0.22	5.0%	10.0%
	4	0.19	0.19	0.20	0.19	0.21	0.21					
100%	5	0.20	0.20	0.21	0.21	0.20	0.21	0.20	0.20	0.20	0.0%	0.0%
	6	0.19	0.19	0.19	0.20	0.20	0.20					

[1] Change in % = (Day 3 – Day 1) ÷ Day 1; change in % = (Day 6 – Day 1) ÷ Day 1. Ear thicknesses ↑ ≥ 25% are considered biologically significant and deemed indicative of a > moderate local dermal irritation response.

Main Test:
Ear thicknesses were measured on Study Day 1 prior to dosing, on Study Day 3 before the third test substance application (48 hours after the initial dose), and on Study Day 6 before sacrifice. As indicated in the Table below, none of the test substance treatments resulted in increases in ear thickness of 25% or more and none of the SIs from the treatment groups was greater than 1.6 (>1.6); therefore, the test substance was not considered irritating.

The Positive Control, 25% HCA, had an ear thickness change of greater than 25% and a SI of greater than 1.6 on Study Day 6, which is consistent with historical results when using 25% HCA in the vehicle AOO, as stated in the study report.

Conc. Level	Mean Ear Thickness (mm)			Change (%)[1]		SI ± S.D.	Body Weight and Body Change (mean ± S.D.; grams)		
	Predoing (Day 1)	48-hr (Day 3)	Study End (Day 6)	Day 3 – Day 1	Day 6 – Day 1		Day 1	Day 6	B.W. Change
Naïve Control	0.20	0.20	0.20	0.0%	0.0%	1.0 ± 0.6	20.5 ± 0.4 (n = 5)	21.1 ± 0.2 (n = 5)	0.6 ± 0.5 (n = 5)
Vehichle Control (AOO)	0.20	0.20	0.21	0.0%	5.0%	1.0 ± 0.5	21.2 ± 0.9 (n = 5)	21.9 ± 1.1 (n = 5)	0.7 ± 0.6 (n = 5)
Positivel Control (25% HCA)	0.20	0.25	0.26	25.0%*	30.0%*	2.7# ± 0.5	21.2 ± 0.6 (n = 5)	22.1 ± 0.8 (n = 5)	0.9 ± 0.4 (n = 5)
25% (v/v in AOO)	0.20	0.21	0.20	5.0%	0.0%	1.0 ± 0.2	21.2 ± 1.1 (n = 5)	22.2 ± 0.8 (n = 5)	1.0 ± 0.8 (n = 5)
50% (v/v in AOO)	0.20	0.21	0.21	5.0%	5.0%	0.8 ± 0.2	20.2 ± 1.3 (n = 5)	20.5 ± 1.4 (n = 5)	0.3 ± 0.3 (n = 5)
100%	0.20	0.21	0.21	5.0%	5.0%	0.8 ± 0.2	20.9 ± 0.1 (n = 5)	22.0 ± 0.3 (n = 5)	1.1 ± 0.2 (n = 5)

[1] Change in % = (Day 3 – Day 1) ÷ Day 1 or = (Day 6 – Day 1) ÷ Day 1.
* Ear thicknesses ↑ ≥ 25% is indicative of a positive dermal irritation response (> moderate).
# A SI ≥1.6 indicates a sensitizing response.